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(71) Applicant *(for all designated States except US)*: THE ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND [US/US]; 1430 Tulane Avenue, New Orleans, LA 70112 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: SCHALLY, Andrew, V. [US/US]; 5025 Kawanee Avenue, Metairie, LA 70006 (US). ZARANDI, Marta [HU/HU]; Danko P. u. 11, H-6723 Szeged H. (HU). TOTH, Katalin [HU/US]; 3500 Houma Boulevard #205, Metairie, LA 70006 (US).

(74) Agent: BEHR, Omri, M.; 325 Pierson Avenue, Edison, NJ 08837 (US).

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(57) Abstract

There A peptide selected from the group having the formulae: α) X-R^1-R^2-R^3-R^4-R^5-R^6-Thr-R^8-R^9-R^{10}-R^{11}-R^{12}-Val-Leu-R^{15}-Gln-Leu-Ser-R^{19}-R^{20}-R^{21}-Leu-Leu-Gln-Asp-Ile-R^{27}-R^{28}-R^{29}, wherein X is H, Ac, Aqc, IAc, BrProp, For, Ibu, Nac, 2-Nac, 1- or 2-Npt, 1- or 2-Npr, PhAc, Fpr, or any other aromatic or nonpolar acyl group, R^1 is Tyr, His or Phe(Y), in which Y=H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3, R^2 is D-Arg, D-Cit, D-Har, D-Lys,D-Tic or D-Orn, R^3 is Asp, D-Asp, Ala, D-Ala or Gly, R^4 is Ala, Abu or Gly, R^5 is Ile, Ala or Gly, R^6 is Phe, Tic, Ala, Pro, Tpi, Nal or Phe(Y), in which Y=H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3, R^8 is Asn, Gln, Ser, Thr, Val, Leu, Ile, Ala, D-Ala, D-Asn, D-Gln, D-Thr, D-Leu, Abu, D-Abu, Nle, or Aib, R^9 is Ser, R^{10} is Tyr or Phe(Y), in which Y=H, F, Cl, Br, NO_2, CH_3 or OCH_3, R^{11} is Arg, D-Arg, or Cit, R^{12} is Lys, D-Lys, Cit, D-Cit, Orn, D-Orn, Nle or Ala, R^{15} is Gly, Ala, Abu or Gln, R^{19} is Ala or Abu, R^{20} is Arg, D-Arg or Cit, R^{21} is Lys, D-Lys, Orn or Cit, R^{27} is Met, Nle or Abu, R^{28} is Ser, Asn, Asp, Ala or Abu, R^{29} is Agm, Arg-NH_2, Arg-OH, Cit-NH_2, Cit-OH, Har-NH_2 or Har-OH, and β) X-A^1-B^2-A^3-R^4-R^5-R^6-Thr-A^8-Ser-R^{10}-R^{11}-B^{12}-Val-Leu-R^{15}-A^{16}-A^{17}-Ser-R^{19}-B^{20}-B^{21}-Leu-Leu-Gln-A^{25}-Ile-R^{27}-R^{28}-B^{29}, wherein a lactam bridge is formed between any one of the pairs of positions 1,2; 2,3; 8,12; 16,20; 17-21; 21,25; 25,29 or both 8,12 and 21,25 positions, and X is H, Ac, Aqc, IAc, BrProp, For, Ibu, Nac, 2-Nac, 1- or 2-Npt, 1- or 2-Npr, PhAc, Fpr, or any other aromatic or nonpolar acyl group, A is Glu, D-Glu, Gln, Asp, D-Asp, Asn, Abu, Leu, Tyr, His, Phe(Y), in which Y=H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3, Ser, Thr, Val, Ile, Ala, D-Ala, D-Asn, D-Gln, D-Thr, D-Leu, Abu, D-Abu, Nle, or Aib, B is Lys, D-Lys, Arg, D-Arg, Orn, D-Orn, or Agm; R^4 is Ala, Abu or Gly, R^5 is Ile, Ala or Gly, R^6 is Phe, Tic, Tpi, Nal or Phe(Y), in which Y=H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3, R^{10} is Tyr or Phe(Y), in which Y=H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3, R^{15} is Gly, Ala, Abu or Gln, R^{27} is Met, Nle or Abu, R^{28} is Ser, Asn, Asp, Ala or Abu, and pharmaceutically acceptable salts thereof.

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hGH-RH(1-29)NH2 ANALOGUES HAVING ANTAGONISTIC ACTIVITY

FIELD OF INVENTION

This invention was made in part with Government support from the Medical Research Service of the Veterans Affairs Department. The Government has certain rights in this application.

The present invention relates to novel synthetic peptides which inhibit the release of growth hormone from the pituitary in mammals, and to therapeutic compositions containing these novel peptides.

BACKGROUND OF THE INVENTION

Growth Hormone ("GH") is a peptide having 191 amino acids which stimulates the production of numerous different growth factors, e.g. IGF-I and so promotes growth of numerous tissues (skeleton, connective tissue, muscle and viscera) and physiological activities (raising nucleic acid and protein synthesis and lipolysis, but lowering urea secretion).

Release of GH is under the control of releasing and inhibiting factors secreted by the hypothalamus. The primary releasing factor is growth hormone releasing hormone ("GH-RH"); human growth hormone-releasing hormone ("hGH-RH") is a peptide having 44 amino acids. The novel peptides of the present invention relate to analogues of hGH-RH having only residues 1 through 29 ("hGH-RH(1-29)NH_2"), i.e., to analogues of the peptide which has the amino acid sequence:

Tyr-Ala-Asp-Ala-Ile^5-Phe-Thr-Asn-Ser-Tyr^{10}-Arg-Lys-Val-Leu-Gly^{15}-Gln-Leu-Ser-Ala-Arg^{20}-Lys-Leu-Leu-Gln-Asp^{25}-Ile-Met-Ser-Arg^{29}-NH_2

GH has been implicated in several diseases. One disease in which GH is involved is acromegaly, in which excessive levels of GH are present. The abnormally enlarged facial and extremity bones of this disease can be

treated by administering a GH-RH antagonist.

Further diseases involving GH are diabetic retinopathy and diabetic nephropathy. The damage to the retina and kidneys respectively in these diseases, believed to be due to GH, results in blindness or reduction in kidney function. This damage however can be prevented or slowed by administration of an effective GH-RH antagonist.

In an effort to intervene in these disease and other conditions, some investigators have attempted to control GH levels by using somatostatin, one inhibitor of GH release. However, somatostatin, if administered alone, does not suppress GH or IGF-I levels to a desired degree. If administered in combination with a GH-RH antagonist, somatostatin would improve suppression of IGF-I levels much better.

Other workers have investigated various modifications of GH-RH to elucidate the relationship of the structure of GH-RH to its activity in an effort to provide synthetic congeners with improved agonistic or antagonistic properties. (Synthesis may be by solid phase method, described in US Patent 4,914,189, or in liquid phase, as described in US Patent 4,707,541.) Thus, in one study, it was found that synthesizing GH-RH without its N-terminus residue -- i.e., forming hGH-RH(2-44) -- results in an analogue having GH releasing activity which is only 0.1% that of GH-RH. By contrast, synthesizing a GH-RH analogue without its residues 30 through 44 -- i.e., synthesizing hGH-RH(1-29)NH_2 -- results in an analogue which retains 50% or more of the potency of native hGH-RH. Synthesizing even shorter analogues -- e.g., GH-RH(1-28)NH_2 or GH-RH(1-27)NH_2 -- resulted in substantially lower bioactivity. These results indicate that sequence 1-29 is important to the bioactivity of GH-RH.

In another study, it was found that acetylating the N-terminus amino acid residue of GH-RH or replacing it with a D-isomer -- thus forming [Ac-Tyr^1]GH-RH or [D-Tyr^1]GH-RH-- lowers the ability of the analogues to release GH to 2-3% that of GH-RH. These analogues also have less affinity in vitro for GH-RH binding sites. By contrast, acetylation of the alpha amino group of residue 1 in hGH-RH(1-29)NH_2 -- thus forming [AcTyr^1]hGH-RH(1-29)NH_2 -- is found to raise the in vivo potency over that of GH-RH by ten fold or more.

In further studies, it was found that [Ac-Tyr^1,D-Arg^2]hGH-RH(1-29) NH_2 antagonizes the activation of rat anterior pituitary adenylate cyclase by hGH-RH(1-29)NH_2. The same peptide was found to block the action of GH-RH on its receptors in the pituitary and hypothalamus, and to inhibit the pulsatile growth hormone secretion.

Several reported modifications to GH-RH have resulted in agonistic activity. US Patent 4,659,693 discloses agonists of hGH-RH(1-29) having the formula:

R^1-R^2-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-R^{27}-Ser-Arg-NH_2,

wherein R^1 is H, Tyr or His; R^2 may be various residues; and R^{27} is Nle. These agonists are said to stimulate release of growth hormone releasing factor ("GRF") and so to be suitable in pharmaceutical compositions. ("GRF" is merely a synonym for GH-RH, and the latter abbreviation is used hereinafter, despite use of GRF in US 4,659,693 and other publications.)

US Patent 4,914,189 discloses other analogues of GH-RH which are agonists. In these agonists, the N-terminus group Q^1CO-, where Q^1 signifies certain omega or alpha-omega substituted alkyl groups, may be Tyr or des-amino-Tyr; the C-terminus group NH-Q^2, where Q^2 signifies certain lower omega-guanidino-alkyl groups, may be Agm; and R^{27} may be Nle. These

analogues are said to be extremely potent stimulants of GH release and to enjoy high resistance to in vivo enzymatic degradation due to the omega-guanidino-lower alkyl group at the C-terminus.

Published application WO 91/16923 reviews earlier attempts to alter the secondary structure of hGH-RH by modifying its amino acid sequence. These earlier attempts include: replacing Tyr^1, Ala^2, Asp^3 or Asn^8 with their D-isomers; replacing Ser^9 with Ala to enhance amphilicity of the region; and replacing Asn^8 with L- or D-Ser, D-Arg, Asn, Thr, Gln or D-Lys. Certain of these modifications are said to enhance GH releasing activity. WO 91/16923 also states that replacing Asn^8 with Ala induces an enormous increase in GH releasing activity. The peptides said to have this benefit have the formula:

$$[R^1,R^2,Ala^8,R^{15}, Nle^{27}]hGH\text{-}RH(1\text{-}29)\text{-}NH_2,$$

where R^1 is Dat or A-R^1, where A is lower acyl or benzyl and R^1 includes Tyr and His; R^2 is Ala, D-Ala or N-Me-D-Ala (N-Methyl-D-Ala); and R^{15} may include Gly, Ala or Aib. One preferred embodiment has $R^{8,9,15}$ as Ala. It is noted that R^8 in this publication is never Asn. Pharmaceutical compositions for enhancing growth are further disclosed.

European Patent Application Serial No. 0 413 839 A, filed August 22, 1989, assigned to the same assignee as the present application, discloses analogues of hGH-RH(1-29)-NH_2 said to have enhanced release of GH. The analogues of this application replace residues 1, 2, 8, 12, 15, 27, 28 and 29 as follows: R^1 may be Tyr or Dat; R^2 may be L or D Ala; R^8 may be Asn or Ser; R^{12} may be L or D isomers of Lys, Arg or Orn; R^{15} may be Gly or Ala; R^{27} may be Nle; R^{28} may be Asp, Asn or Ser; and R^{29} may be Agm. However, residue 6 is never replaced: it is always Phe.

Yet another modification of hGH-RH was disclosed in US Patent 5,183,660, where GH-RH was conjugated with polyethylene glycol

derivatives. The resulting conjugate was said to exhibit decreased antigenicity, delay in biological clearance in vivo and physiological activity over a longer time.

In several of these investigations, it was found that variants of the hGH-RH agonistic analogues had antagonistic, rather than agonistic, activity. Thus, in US 4,659,693 (where R^2 may be certain D-Arg residues substituted with alkyl groups), when R^1 is H, the hGH-RH analogues are said to act as antagonists. Similarly, in WO 91/16923, discussed above, if R^2 in the analogues is D-Arg, and R^8, R^9, and R^{15} are substituted as indicated above, antagonistic activity is said to result. These antagonistic peptides are said to be suitable for administration as pharmaceutical compositions to treat conditions associated with excessive levels of GH, e.g., acromegaly.

The antagonistic activity of the hGH-RH analogue "[Ser^9-Ψ[CH_2-NH]-Tyr^{10}]hGH-RH(1-29)" of US Patent 5,084,555 was said to result from the pseudopeptide bond (i.e., a peptide bond reduced to a [CH_2-NH] linkage) between the R^9 and R^{10} residues. (It is noted that although this patent employed the seemingly redundant "Ψ[CH_2-NH]" formula for the pseudopeptide bond, actually only one such linkage had been introduced into the peptide.) However, the antagonistic properties of [Ser^9-Ψ[CH2-NH]-Tyr^{10}] hGH-RH(1-29) were said to be inferior to a conventional antagonist, [N-Ac-Tyr^1, D-Arg^2]GH-RH(1-29)-NH_2.

US Patent 5,084,442 discloses cyclic lactam analogues of GH-RH which are agonists. The introduction of lactam bridge into peptides or proteins is known to reduce the number of possible conformations. Incorporation of constraints into biologically active peptides may improve receptor binding and result in analogs which have enhanced or prolonged biological activity.

SUMMARY OF THE INVENTION

There is provided a novel series of synthetic analogues of hGH-RH(1-29)NH_2. These analogues inhibit the activity of endogenous hGH-RH, and therefore prevent the release of growth hormone. This inhibition is believed to result from replacement of various amino acids and acylation with aromatic or nonpolar acids at the N-terminus of GH-RH(1-29)NH_2. The analogues exhibit prolonged antagonistic duration.

Specifically, the invention relates to peptides comprising the formulae:

a) X-R^1-R^2-R^3-R^4-R^5-R^6-Thr-R^8-R^9-R^{10}-R^{11}-R^{12}-Val-Leu-R^{15}-Gln-Leu-Ser-R^{19}-R^{20}-R^{21}-Leu-Leu-Gln-Asp-Ile-R^{27}-R^{28}-R^{29}

wherein X is H, Ac, Aqc, IAc, BrProp, For, Ibu, Nac, 2-Nac, 1- or 2-Npt, 1- or 2-Npr, PhAc, Fpr, or any other aromatic or nonpolar acyl group,

R^1 is Tyr, His or Phe(Y), in which Y = H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3,

R^2 is D-Arg, D-Cit, D-Har, D-Lys, D-Tic or D-Orn,

R^3 is Asp, D-Asp, Ala, D-Ala or Gly,

R^4 is Ala, Abu or Gly,

R^5 is Ile, Ala or Gly,

R^6 is Phe, Tic, Ala, Pro, Tpi, Nal or Phe(Y), in which Y = H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3,

R^8 is Asn, Gln, Ser, Thr, Val, Leu, Ile, Ala, D-Ala, D-Asn, D-Gln, D-Thr, D-Leu, Abu, D-Abu, Nle, or Aíb,

R^9 is Ser

R^{10} is Tyr or Phe(Y), in which Y = H, F, Cl, Br, NO_2, CH_3 or OCH_3,

R^{11} is Arg, D-Arg, or Cit,

R^{12} is Lys, D-Lys, Cit, D-Cit, Orn, D-Orn, Nle or Ala,

R^{15} is Gly, Ala, Abu or Gln,

R^{19} is Ala or Abu,

R^{20} is Arg, D-Arg or Cit,

R^{21} is Lys, D-Lys, Orn or Cit,

R^{27} is Met, Nle or Abu,

R^{28} is Ser, Asn, Asp, Ala or Abu,

R^{29} is Agm, Arg-NH_2, Arg-OH, Cit-NH_2, Cit-OH, Har-NH_2 or Har-OH, and pharmaceutically acceptable salts thereof.

Among the preferred embodiment are peptides wherein X is PhAc or Ibu, R^1 is Tyr or His, R^2 is D-Arg or D-Cit, R^3 is Asp, R^4 is Ala, R^5 is Ile, R^6 is Phe(pCl) or Nal, R^8 is Asn, D-Asn, D-Thr or Abu, R^{10} is Tyr or Phe(pCl), R^{11} is Arg, R^{12} is Lys or D-Lys, R^{15} is Abu or Ala, R^{19} is Ala or Abu, R^{20} is Arg, R^{21} is Lys, R^{27} is Nle, R^{28} is Ser , Asp, or Abu, R^{29} is Agm or Arg-NH_2.

Six very preferred embodiments have the formulae:

$PhAc^0$-Tyr^1-D-Arg^2-Asp^3-Ala^4-Ile^5-$Phe(pCl)^6$-Thr^7-Asn^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Asp^{28}-Agm Peptide 5

$PhAc^0$-Tyr^1-D-Arg^2-Asp^3-Ala^4-Ile^5-$Phe(pCl)^6$-Thr^7-Asn^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-Agm Peptide 8

Ibu^0-Tyr^1-D-Arg^2-Asp^3-Ala^4-Ile^5-$Phe(pCl)^6$-Thr^7-Asn^8-Ser^9-$Phe(pCl)^{10}$-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-Agm Peptide 6

$PhAc^0$-Tyr^1-D-Arg^2-Asp^3-Ala^4-Ile^5-$Phe(pCl)^6$-Thr^7-Asn^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-Arg^{29}-NH_2 Peptide 9

$PhAc^0$-Tyr^1-D-Arg^2-Asp^3-Ala^4-Ile^5-$Phe(pCl)^6$-Thr^7-Abu^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-Arg^{29}-NH_2 Peptide 11

$PhAc^0$-Tyr^1-D-Arg^2-Asp^3-Ala^4-Ile^5-$Phe(pCl)^6$-Thr^7-Abu^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Abu^{28}-Arg^{29}-NH_2 Peptide 13

Under well-established convention, these may be abbreviated as follows:

[PhAc^0,D-Arg^2, Phe(pCl)^6, Ala^{15}, Nle^{27}, Asp^{28}]hGH-RH(1-28)Agm Peptide 5

[Ibu^0,D-Arg^2, Phe(pCl)$^{6, 10}$, Abu^{15}, Nle^{27}]hGH-RH(1-28)Agm Peptide 6

[PhAc^0,D-Arg^2, Phe(pCl)^6, Abu^{15}, Nle^{27}]hGH-RH(1-28)Agm Peptide 8

[PhAc^0,D-Arg^2, Phe(pCl)^6, Ala^{15}, Nle^{27}]hGH-RH(1-29)-NH_2 Peptide 9

[PhAc^0,D-Arg^2,Phe(pCl)^6,Abu^8,Ala^{15},Nle^{27},hGH-RH(1-29)NH_2

Peptide 11

[PhAc^0,D-Arg^2, Phe(pCl)^6, Abu$^{8, 28}$, Ala^{15}, Nle^{27}]hGH-RH(1-29)-NH_2

Peptide 13

β) X-A^1-B^2-A^3-R^4-R^5-R^6-Thr-A^8-Ser-R^{10}-R^{11}-B^{12}-Val-Leu-R^{15}-A^{16}-A^{17}-Ser-R^{19}-B^{20}-B^{21}-Leu-Leu-Gln-A^{25}-Ile-R^{27}-R^{28}-B^{29}

wherein lactam bridge is formed between positions 1,2; 2,3; 8,12; 16,20; 17-21; 21,25; 25,29 or both 8,12 and 21,25 positions and

X is H, Ac, Aqc, IAc, BrProp, For, Ibu, Nac, 2-Nac, 1- or 2-Npt, 1- or 2-Npr, PhAc, Fpr or any other aromatic or nonpolar acyl group,

A is Glu, D-Glu, Gln, Asp, D-Asp, Asn, Abu, Leu, Tyr, His, Phe(Y), in which Y = H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3, Ser, Thr, Val, Ile, Ala, D-Ala, D-Asn, D-Gln, D-Thr, D-Leu, Abu, D-Abu, Nle, or Aib,

B is Lys, Lys-OH, Lys-NH_2, D-Lys, D-Lys-OH, D-Lys-NH_2, Arg, Arg-OH, Arg-NH_2, D-Arg, D-Arg-OH, D-Arg-NH_2, Orn, Orn-OH, Orn-NH_2, D-Orn, D-Orn-OH, D-Orn-NH_2, Har, Har-OH, Har-NH_2, or Agm

R^4 is Ala, Abu or Gly,

R^5 is Ile, Ala or Gly,

R^6 is Phe, Tic, Tpi, Nal or Phe(Y), in which Y = H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3,

R^{10} is Tyr or Phe(Y), in which Y = H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3,

R^{15} is Gly, Ala, Abu or Gln,

R^{27} is Met, Nle or Abu,

R^{28} is Ser, Asn, Asp, Ala or Abu,

and pharmaceutically acceptable salts thereof.

Among the preferred embodiment are peptides wherein X is PhAc or Ibu, A^1 is Tyr, His, Glu, Asp or D-Asp, B^2 is D-Arg, D-Cit or D-Lys, A^3 is Asp, R^4 is Ala, R^5 is Ile, R^6 is Phe(pCl) or Nal, A^8 is Asn, D-Asn, Abu, Asp, D-Asp, Glu or D-Glu, R^{10} is Tyr or Phe(4-Cl), R^{11} is Arg, B^{12} is Lys or D-Lys, R^{15} is Abu or Ala, A^{16} is Gln or Glu, A^{17} is Leu or Glu, R^{19} is Ala or Abu, B^{20} is Arg or Lys, B^{21} is Lys, A^{25} is Asp or Glu, R^{27} is Nle, R^{28} is Ser, Asp, or Abu, B^{29} is Agm, Arg-NH_2 or Orn-NH_2 and lactam bridge is formed between positions 8,12; 17,21 or both 8,12 and 21,25 positions. Five very preferred embodiments have the formulae:

cyclo^{8,12}[PhAc^0-Tyr^1-D-Arg^2-Asp^3-Ala^4-Ile^5-Phe(pCl)^6-Thr^7-Glu^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-Arg-NH_2 Peptide 17

cyclo^{17,21}[PhAc^0-Tyr^1-D-Arg^2-Asp^3-Ala^4-Ile^5-Phe(pCl)^6-Thr^7-Ser^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Glu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-Arg-NH_2 Peptide 18

cyclo$^{8,12;\ 21,25}$[PhAc^0-Tyr^1-D-Arg^2-Asp^3-Ala^4-Ile^5-Phe(pCl)^6-Thr^7-Glu^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Glu^{25}-Ile^{26}-Nle^{27}-Ser^{28}-Agm Peptide 21

cyclo$^{8,12;\ 21,25}$[PhAc^0-Tyr^1-D-Arg^2-D-Asp^3-Ala^4-Ile^5-Phe(pCl)^6-Thr^7-Glu^8-Ser^9-Tyr^{10}-Arg^{11}-D-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Glu^{25}-Ile^{26}-Nle^{27}-Ser^{28}-Arg^{29}-NH_2 Peptide 22

cyclo$^{8,12;\ 21,25}$[PhAc^0-Tyr^1-D-Arg^2-Asp^3-Ala^4-Ile^5-Phe(pCl)^6-Thr^7-Glu^8-Ser^9-Tyr^{10}-Arg^{11}-D-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Glu^{25}-Ile^{26}-Nle^{27}-Ser^{28}-Arg^{29}-NH_2 Peptide 23

Under well-established convention, these may be abbreviated as follows:

cyclo^{8,12}[PhAc^0,D-Arg^2,Phe(pCl)^6,Glu^8,Ala^{15},Nle^{27}]hGH-RH(1-29)-NH_2 Peptide 17

cyclo^{17,21}[PhAc^0,D-Arg^2,Phe(pCl)^6,Ser^8,Ala^{15},Glu^{17},Nle^{27}]hGH-RH(1-29)-NH_2 Peptide 18

cyclo$^{8,12;\ 21,25}$[PhAc^{0},D-Arg^{2},Phe(pCl)^{6},Glu^{8,25},Abu^{15},Nle^{27}] hGH-RH(1-28)Agm Peptide 21

cyclo$^{8,12;\ 21,25}$[[PhAc^{0},D-Arg^{2},D-Asp^{3},Phe(pCl)^{6},Glu^{8,25},D-Lys^{12}, Ala^{15}, Nle^{27}]hGH-RH(1-29)-NH_2 Peptide 22

cyclo$^{8,12;\ 21,25}$[[PhAc^{0},D-Arg^{2}, Phe(pCl)^{6}, Glu^{8,25}, D-Lys^{12}, Ala^{15}, Nle^{27}] hGH-RH(1-29)-NH_2 Peptide 23

It is noted that the amino acid residues from 30 through 44 of the native GH-RH molecule do not appear to be essential to activity; nor does their identity appear to be critical. Therefore, it appears that the addition of some or all of these further amino acid residues to the C-terminus of the hGH-RH(1-29)-NH_2 analogues of the present invention will not affect the efficacy of these analogues as GH-RH antagonists. If some or all of these amino acids were added to the C-terminus of the hGH-RH(1-29)-NH_2 analogues, the added amino acid residues could be the same as residues 30 through 44 in the native hGH-RH sequence or reasonable equivalents.

Synthetic Methods.

The synthetic peptides are synthesized by a suitable method such as by exclusive solid phase techniques, by partial solid-phase techniques, by fragment condensation or by classical solution phase synthesis.

When the analogues of this invention are synthesized by solid-phase method, the C-terminus residue (here, R^{29}) is appropriately linked (anchored) to an inert solid support (resin) while bearing protecting groups for its alpha amino group (and, where appropriate, for its side chain functional group). After completion of this step, the alpha amino protecting group is removed from the anchored amino acid residue and the next amino acid residue, R^{28}, is added having its alpha amino group (as well as any appropriate side chain functional group) suitably protected, and so forth. The N-terminus protecting groups are removed after each residue is added, but the side chain protecting groups are not yet removed. After all the desired amino

acids have been linked in the proper sequence, the peptide is cleaved from the support and freed from all side chain protecting group(s) under conditions that are minimally destructive towards residues in the sequence. This is be followed by a careful purification and scrupulous characterization of the synthetic product, so as to ensure that the desired structure is indeed the one obtained.

It is particularly preferred to protect the alpha amino function of the amino acids during the coupling step with an acid or base sensitive protecting group. Such protecting groups should have the properties of being stable in the conditions of peptide linkage formation, while being readily removable without destruction of the growing peptide chain and without racemization of any of the chiral centers contained therein. Suitable alpha amino protecting groups are Boc and Fmoc.

Medical Applications.

The hGH-RH antagonist peptides, or salts of these peptides, may be formulated in pharmaceutical dosage forms containing effective amounts thereof and administered to humans or animal for therapeutic or diagnostic purposes. The peptides may be used to suppress GH levels and to treat conditions associated with excessive levels of GH, e.g., diabetic retinopathy and nephropathy, and acromegaly. Also provided are methods for treating these diseases by administration of a composition of the invention to an individual needing such treatment. The main uses of GH-RH antagonists are however,in the field of cancer, for example human cancers of the breast, lung, colon, brain, and pancreas where the receptors for IGF-I are present.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a plot of volume changes of SW 1990 human pancreatic cancers in nude mice during treatment with certain GH-RH antagonists against days of treatment.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

A. Abbreviations

The nomenclature used to define the peptides is that specified by the IUPAC-IUB Commissioner on Biochemical Nomenclature wherein, in accordance with conventional representation, the amino group at the N-terminus appears to the left and the carboxyl group at the C-terminus appears to the right. The term "natural amino acid" as used herein means one of the common, naturally occurring L-amino acids found in naturally occurring proteins: Gly, Ala, Val, Leu, Ile, Ser, Thr, Lys, Arg, Asp, Asn, Glu, Gln, Cys, Met Phe, Tyr, Pro, Trp and His. When the natural amino acid residue has isomeric forms, it is the L-form of the amino acid that is represented herein unless otherwise expressly indicated.

Non-coded amino acids, or amino acid analogues, are also incorporated into the GH-RH antagonists. ("Non-coded" amino acids are those amino acids which are not among the approximately 20 natural amino acids found in naturally occurring peptides.) Among the non-coded amino acids or amino acid analogues which may be used in the hGH-RH antagonist peptides are the following: by Abu is meant alpha amino butyric acid, by Agm is meant agmatine (1-amino-4-guanidino-butane), by Aib meant alpha amino isobutyric acid, by Har is meant homoarginine, by hPhe is meant homo-phenylalanine, by Nal is meant 2-naphthyl-alanine, by Nle is meant norleucine, and by Tic is meant 1,2,3,4-tetrahydroisoquinoline carboxilic acid. When these non-coded amino acids, or amino acid analogues, have isomeric forms, it is the L-form of the amino acid that is represented unless otherwise expressly indicated.

Abbreviations used herein are:

Abu	α-aminobutyric acid
Ac	acetyl
AcOH	acetic acid

Ac_2O	acetic anhydride
Agm	agmatine (1-amino-4-guanidino-butane)
Aib	α-aminoisobutyric acid
Aqc	anthraquinone-2-carbonyl
BHA	benzhydrylamine
Boc	tert.butyloxycarbonyl
BOP	benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
BrProp	bromopropionyl
cHx	cyclohexyl
Cit	citrulline (2-amino-5-ureidovaleric acid)
DCM	dichloromethane
DIC	N,N'-diisopropylcarbodiimide
DIEA	diisopropylethylamine
DMF	dimethylformamide
Fpr	3-phenylptopionyl
GH	growth hormone
GH-RH	GH releasing hormone
Har	homoarginine
HBTU	2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexaflourophosphate
hGH-RH	human GH-RH
HOBt	1-hydroxybenzotriazole
hPhe	homophenylalanine
HPLC	high performance liquid chromatography
IAc	iodoacetyl
Ibu	isobutyryl
MeOH	methanol
MeCN	acetonitrile
MBHA	para-methylbenzhydrylamine
Nac	1-naphthylacetyl

2-Nac	2-naphthylacetyl
Nal	2-naphthylalanine
NMM	N-methylmorpholine
Npr	naphthylpropionyl
Npt	naphthoyl
Phe(pCl)	para-chloro-phenylalanine
PhAc	phenylacetyl
rGH-RH	rat GH-RH
RP-HPLC	reversed phase HPLC
SPA	sulfophenoxy
TFA	trifluoroacetic acid
Tic	1,2,3,4-tetrahydroisoquinoline
Tos	para-toluenesulfonyl
Tpi	2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-3-carboxylic acid
Tyr(Me)	tyrasine methylether
Z	benzyloxycarbonyl

B. The GH-RH Analogues

The hGH-RH analogues of the present invention were designed to increase the affinities of the peptides to the receptor, to improve metabolic stability and to maximize the amphiphilic secondary structure of the molecules. Many of these analogués cause very effective and long lasting inhibition of the GH release stimulated by hGH-RH(1-29)NH_2 in vitro and in vivo.

The following embodiments are specially preferred as having remarkable bioactivity:

[Ibu^0,D-Arg^2,Phe(pCl)^6,Abu^{15},Nle^{27},Cit^{29}]hGH-RH(1-29)-NH_2

Peptide # 1

[Ibu^0,D-Arg^2,Phe(pCl)^6, Abu^{8,15},Nle^{27},Cit^{29}]hGH-RH(1-29)-NH$_2$

Peptide # 2

[PhAc^0,D-Arg^2,Phe(pCl)^6,Abu^{8,15},Nle^{27},Cit^{29}]hGH-RH(1-29)-NH$_2$

Peptide # 3

[Ac-Nal^0,D-Arg^2,Phe(pCl)^6,Abu^{15},Nle^{27}]hGH-RH(1-28)Agm

Peptide # 4

[PhAc^0,D-Arg^2,Phe(pCl)^6,Ala^{15},Nle^{27},Asp^{28}]hGH-RH(1-28)Agm

Peptide # 5

[Ibu^0,D-Arg^2,Phe(pCl)^{6,10},Abu^{15},Nle^{27}]hGH-RH(1-28)Agm

Peptide # 6

[Ibu^0,D-Arg^2,Phe(pCl)^6,D-Thr^8,Abu^{15},Nle^{27}]hGH-RH(1-28)Agm

Peptide # 7

[PhAc^0,D-Arg^2,Phe(pCl)^6,Abu^{15},Nle^{27}]hGH-RH(1-28)Agm

Peptide # 8

[PhAc^0,D-Arg^2,Phe(pCl)^6,Ala^{15},Nle^{27}]hGH-RH(1-29)-NH$_2$

Peptide # 9

[PhAc^0,Tyr(Me),D-Arg^2,Ala^{15},Nle^{27}]hGH-RH(1-29)-NH$_2$

Peptide # 10

[PhAc^0,D-Arg^2,Phe(pCl)^6,Abu^8,Ala^{15},Nle^{27}],hGH-RH(1-29)NH$_2$

Peptide # 11

[PhAc^0,D-Arg^2,Nal^6,Abu^{8,28},Ala^{15},Nle^{27}]hGH-RH(1-29)NH$_2$

Peptide # 12

[PhAc^0,D-Arg^2,Phe(pCl)^6,Abu^{8,28},Ala^{15},Nle^{27}]hGH-RH(1-29)NH$_2$

Peptide # 13

[PhAc^0,D-Arg^2,Phe(pCl)^6,Abu^8,D-Lys^{12},Ala^{15},Nle^{27}]hGH-RH(1-29)NH$_2$

Peptide # 14

[PhAc^0,D-Arg^2,Phe(pCl)^6,Orn^{12,21},Abu^{15},Nle^{27}]hGH-RH(1-28)Agm

Peptide # 15

[Ibu^0,D-Arg^2,Phe(pCl)^6,Orn^{12,21},Abu^{15},Nle^{27}]hGH-RH(1-28)Agm

Peptide # 16

cyclo ^{8,12}[PhAc^0,D-Arg^2,Phe(pCl)^6,Glu^8,Ala^{15},Nle^{27}]hGH-RH(1-29)NH_2
Peptide # 17
cyclo ^{17,21}[PhAc^0,D-Arg^2,Phe(pCl)^6,Ser^8,Ala^{15},Glu^{17},Nle^{27}]hGH-RH(1-29)NH_2
Peptide # 18
cyclo^{25,29}[PhAc^0,D-Arg^2,Phe(pCl)^6,Abu^8,Ala^{15},D-Asp^{25},Nle^{27},Orn^{29}]hGH-RH(1-29)NH_2 Peptide # 19
cyclo^{25,29}[PhAc^0,D-Arg^2,Nal^6,Abu^8,Ala^{15},D-Asp^{25},Nle^{27},Orn^{29}]hGH-RH(1-29)NH_2 Peptide # 20
cyclo$^{8,12;21,25}$[PhAc^0,D-Arg^2,Phe(pCl)^6,Glu^{8,25},Abu^{15}, Nle^{27}]hGH-RH(1-28)Agm Peptide # 21
cyclo$^{8,12;21,25}$[PhAc^0,D-Arg^2,D-Asp^3,Phe(pCl)^6,Glu^{8,25},D-Lys^{12},Ala^{15},Nle^{27}]hGH-RH(1-29)NH_2 Peptide # 22
cyclo$^{8,12;21,25}$[PhAc^0,D-Arg^2,Phe(pCl)^6,Glu^{8,25},D-Lys^{12},Ala^{15},Nle^{27}]hGH-RH(1-29)NH_2 Peptide # 23
cyclo$^{8,12;21,25}$[PhAc^0,D-Arg^2,Phe(pCl)^6,Asp^8,D-Lys^{12},Ala^{15},Glu^{25},Nle^{27}]hGH-RH(1-29)NH_2 Peptide # 24
[PhAc^0,D-Arg^2,Tic^6,Abu^{15},Nle^{27}]hGH-RH(1-28)Agm
Peptide # 25
[PhAc^0,D-Tic^2,Phe(pCl)^6,Abu^{15},Nle^{27}]hGH-RH(1-28)Agm
Peptide # 26

C. Method of Preparation

1. Overview of Synthesis

The peptides are synthesized by a suitable method such as by exclusive solid phase techniques, by partial solid-phase techniques, by fragment condensation or by classical solution phase synthesis. For example, the techniques of exclusive solid-phase synthesis are set forth in the textbook "Solid Phase Peptide Synthesis", J.M. Stewart and J.D. Young, Pierce Chem. Company, Rockford, 111, 1984 (2nd. ed.), and M. Bodanszky, "Principles of Peptide Synthesis", SpringerVerlag, 1984. The hGH-RH antagonist peptides are preferably prepared using solid phase

synthesis, such as that generally described by Merrifield, J.Am.Chem.Soc., 85, p. 2149 (1963), although other equivalent chemical syntheses known in the art can also be used as previously mentioned.

The synthesis is carried out with amino acids that are protected at their alpha amino group. Urethane type protecting groups (Boc or Fmoc) are preferably used for the protection of the alpha amino group. The preferred protecting group is Boc.

In solid phase synthesis, the N-alpha-protected amino acid moiety which forms the aminoacyl group of the final peptide at the C-terminus is attached to a polymeric resin support via a chemical link. After completion of the coupling reaction, the alpha amino protecting group is selectively removed to allow subsequent coupling reactions to take place at the amino-terminus, preferably with 50% TFA in DCM when the N-alpha-protecting groupg is Boc. The remaining amino acids with similarly Boc-protected alpha amino groups are coupled stepwise to the free amino group of the preceding amino acid on the resin to obtain the desired peptide sequence. Because the amino acid residues are coupled to the alpha amino group of the C-terminus residue, growth of the synthetic hGH-RH analogue peptides begins at the C terminus and progresses toward the N-terminus. When the desired sequence has been obtained, the peptide is acylated, if appropriate, and it is removed from the support polymer.

Each protected amino acid is used in excess (2.5 or 3 equivalents) and the coupling reactions are usually carried out in DCM, DMF or mixtures thereof. The extent of completion of the coupling reaction is monitored at each stage by the ninhydrin reaction. In cases where incomplete coupling is determined, the coupling procedure is repeated before removal of the alpha amino protecting group prior to the coupling of the next amino acid.

A typical synthesis cycle is shown in Table I.

TABLE I

Protocol for a Typical Synthetic Cycle Using Boc-strategy

Step	Reagent	Mixing Time (min)
1. Deprotection	50% TFA in DCM	5 + 25
	DCM wash	1
	2-propanol wash	1
2. Neutralization	5% DIEA in DCM	1
	DCM wash	1
	MeOH wash	1
	5% DIEA in DCM	3
	MeOH wash	1
	DCM wash (3 times)	1-1
3.A Coupling	3 equiv. Boc-amino acid in DCM or DMF + 3 equiv. DIC or the preformed HOBt ester of the Boc-amino acid	60
	MeOH wash	2
	DCM wash	2
3.4. Acetylation (if appropriate)	Ac_2O in DCM (30%)	10 + 20
	MeOH wash (3 times)	2
	DCM wash (3 times)	2

After completion of the synthesis, the cleavage of the peptide from the resin can be effected using procedures well known in peptide chemistry.

Some of the amino acid residues of the peptides have side chain functional groups which are reactive with reagents used in coupling or deprotection. When such side chain groups are present, suitable protecting groups are joined to these functional groups to prevent from undesirable chemical reactions occurring during the reactions used to form the peptides. The following general rules are followed in selecting a particular side chain protecting group: (a) the protecting group preferably retains its protecting properties and is not split off under coupling conditions, (b) the protecting

group should be stable in conditions for removing the alpha amino protecting group at each step of the synthesis, (c) the side chain protecting group must be removable upon the completion of the synthesis of the desired amino acid sequence, under reaction conditions that will not undesirably alter the peptide chain.

The initial synthetic steps utilized herein are disclosed in US Patent 4,914,189 which is incorporated by reference herein. Reference is particularly made to Examples I through IV therein.

The reactive side chain functional groups are preferably protected as follows: benzyl for Thr and Ser; 2,6-dichlorobenzyloxycarbonyl for Tyr; p-toluene-sulfonyl for Arg; 2-chlorobenzyloxycarbonyl or fluorenylmethyloxy carbonyl for Lys, Orn; and cyclohexyl or fluorenylmethyl for Asp and Glu. The side chains of Asn and Gln are unprotected.

2. Coupling R^{29} to the support Polymer

The hGH-RH antagonist peptides may be synthesized on a variety of support polymers. These support polymers may be amino resins such as amino-methyl resins, benzhydrylamine resins, p-methylbenzhydrylamine resins and the like. Boc-R^{29} is the initial material joined to the support phase, suitably Boc-Arg(Tos)-OH or Boc-Agm.

For the synthesis of peptides having Agm at the C-terminus, it is preferred that the support phase [SP] is an amino methyl resin. The guanidino group of Boc-Agm is joined to the support polymer via a stable but readily cleavable bridging group. It has been found that such a bridge may be readily provided by the sulfonyl phenoxy acetyl moiety. The alpha amino Boc-protected Agm is reacted with the chlorosulfonyl phenoxy acetic acid

$Cl\text{-}SO_2\text{-}\phi\text{-}O\text{-}CH_2\text{-}COOH$

to form

$Boc\text{-}Agm^{29}\text{-}SO_2\text{-}\phi\text{-}O\text{-}CH_2\text{-}COOH.$

This compound is then coupled to the support polymer [SP] using DIC or BOP as activating reagent to yield:

$Boc\text{-}Agm^{29}\text{-}SO_2\text{-}\phi\text{-}O\text{-}CH_2\text{-}CO\text{-}[SP]$

For the synthesis of peptides having Arg-NH_2 at the C-terminus, Boc-Arg(Tos)-OH is coupled to the neutralized BHA or MBHA resin using DIC or BOP as activating reagent.

3. Stepwise Coupling of Amino Acid Residues.

Utilizing the Boc-protected Agm resin (California Peptide Res. Inc.), (or the Boc-Arg(Tos)-resin), the peptide itself may suitably be built up by solid phase synthesis in the conventional manner. The selection of an appropriate coupling reagent is within the skill of the art. Particularly suitable as coupling reagents are N,N'-diisopropyl carbodiimide (DIC) or the BOP carboxyl activating reagent.

Each protected amino acid is coupled in about a three-fold molar excess, with respect to resin-bound aminoacyl residue(s), and the coupling may be carried out in as medium such as DMF: CH_2Cl_2 (1:1) or in DMF or CH_2Cl_2 alone. In cases where incomplete coupling occurs, the coupling procedure is repeated before removal of the alpha amino protecting group. The success of the coupling reaction at each stage of the synthesis is preferably monitored by the ninhydrin reaction.

In case of lactam bridge formation, the side-chain protecting groups (Fmoc and OFm) of Lys or Orn and Asp or Glu respectively of the Boc-protected peptide-resin are selectively removed with 20% piperidine in DMF.

Formation of lactam bridge is achieved on the solid support using HBTU coupling agent.

4. Removal of the Peptide from the Support Polymer.

When the synthesis is complete, the peptide is cleaved from the support phase. Removal of the peptide from the resin is performed by treatment with a reagent such as liquid hydrogen fluoride which also cleaves all remaining side chain protecting groups.

Suitably, the dried and protected peptide-resin is treated with a mixture consisting of 1.0 mL m-cresol and 10 mL anhydrous hydrogen fluoride per gram of peptide-resin for 60 min at 0°C to cleave the peptide from the resin as well as to remove all side chain protecting groups. After the removal of the hydrogen fluoride under a stream of nitrogen and vacuum, the free peptides are precipitated with ether, filtered, washed with ether and ethyl acetate, extracted with 50% acetic acid, and lyophilized.

5. Purification

The purification of the crude peptides can be effected using procedures well known in peptide chemistry. For example, purification may be performed on a MacRabbit HPLC system (Rainin Instrument Co. Inc., Woburn, MA) with a Knauer UV Photometer and a Kipp and Zonen BD40 Recorder using a 10 x 250 mm VYDAC 228TP column packed with C8 silica gel (300 Å pore size, 10 μm particle size) (Rainin Inc.). The column is eluted with a solvent system consisting of (A) 0.1% aqueous TFA and (B) 0.1% TFA in 70% aqueous MeCN in a linear gradient mode (e.g., 30-65% B in 120 min). The eluent is monitored at 220 nm, and fractions are examined by analytical HPLC using a Hewlett-Packard Model HP-1090 liquid chromatograph and pooled to give maximum purity. Analytical HPLC is carried out on a W-Porex C18 reversed-phase column (4.6 x 250 mm, 5 μm particle size, 300 Å pore size) (Phenomenex, Rancho Palos Verdes, CA)

using isocratic elution with a solvent system consisting of (A) and (B) defined above. The peaks are monitored at 220 and 280 nm. The peptides are judged to be substantially (>95%) pure by analytical HPLC. The expected amino acid composition is also confirmed by amino acid analysis.

D. Pharmaceutical Composition

The peptides of the invention may be administered in the form of pharmaceutically acceptable, nontoxic salts, such as acid addition salts. Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, fumarate, gluconate, tannate, maleate, acetate, citrate, benzoate, succinate, alginate, pamoate, malate, ascorbate, tartarate, and the like. Particularly preferred antagonists are salts of low solubility, e.g., pamoate salts and the like. These exhibit long duration of activity.

The compounds of the present invention are suitably administered to subject humans or animals s.c., i.m., or i.v; intranasally or by pulmonary inhalation; or in a depot form (e.g., microcapsules, microgranules, or cylindrical rod like implants) formulated from a biodegradable suitable polymer (such as D,L-lactide-coglycolide), the former two depot modes being preferred. Other equivalent modes of administration are also within the scope of this invention, i.e., continuous drip, depot injections, infusion pump and time release modes such as microcapsules and the like. Administration is in any physiologically acceptable injectable carrier, physiological saline being acceptable, though other carriers known to the art may also be used.

The peptides are preferably administered parenterally, intramuscularly, subcutaneously or intravenously with a pharmaceutically acceptable carrier such as isotonic saline. Alternatively, the peptides may be administered as an intranasal spray with an appropriate carrier or by pulmonary inhalation. One suitable route of administration is a depot form formulated from a

biodegradable suitable polymer, e.g., poly-D,L-lactide-coglycolide as microcapsules, microgranules or cylindrical implants containing dispersed antagonistic compounds.

The amount of peptide needed depends on the mode of administration and the intended result. In general, the dosage range is between 1-100 μg/kg of body weight of the host per day.

E. Therapeutic Uses of GH-RH Antagonists

hGH-RH antagonists can be used in treatment of conditions caused by excess growth hormone, for example acromegaly, which is manifested by an abnormal enlargement of the bones of the face and extremities. The GH-RH antagonists may also be used to treat diabetic nephropathy (the main cause of blindness in diabetics) and diabetic retinopathy, in which damage to the eye and kidney respectively is thought to be due to GH.

The hGH-RH antagonists are designed to block the binding and therefore the action of GH-RH, which stimulates the secretion of GH, which in turn stimulates production of IGF I. GH-RH antagonists may be administered alone or together with somatostatin analogues, a combination which more completely suppresses IGF-I levels. It is advantageous to administer antagonists of GH-RH rather than somatostatin due to the fact that GH-RH antagonists may be utilized in situations where target sites do not have somatostatin receptors.

However, the main applications of GH-RH antagonists are in the field of cancer. This is based on the following considerations: GH-RH antagonists are designed to block the binding and therefore the action of GH-RH, which stimulates the secretion of GH, which in turn stimulates production of insulin-like growth factor I (IGF-I) also called somatomedin-C. The involvement of IGF-I (somatomedin-C) in breast cancer, prostate cancer,

colon cancer, bone tumors and other malignancies is well established, and somatostatin analogues alone do not adequately suppress GH and IGF-I levels. A complete suppression of IGF-I levels or secretion is required for a better inhibition of tumor growth. Autocrine production of IGF-I by various tumors could be also under control of GH-RH and might therefore be inhibited by GH-RH antagonists. GH-RH antagonists might also inhibit the production of IGF-I. A more detailed theoretical background of the applications of GH-RH in the field of oncology (cancer) is as follows: The receptors for IGF-I are present in primary human **breast cancers**, in **lung cancers**, in human **colon cancers,** in human **brain tumors**, and in human **pancreatic cancers**.

The presence of IGF-I receptors in these tumors appears to be related to malignant transformation and proliferations of these cancers. IGF-I can act as endocrine, paracrine or autocrine growth factor for various human cancers, that is the growth of these neoplasms is dependent on IGF-I. GH-RH antagonists by suppressing GH secretion would lower the production of IGF-I. Since IGF-I stimulates growth of these various neoplasms (cancers), the lowering of circulating IGF-I levels should lead to tumor growth inhibition. It is possible that GH-RH antagonists could also lower paracrine or autocrine production of IGF-I by the tumors, which should also lead to inhibition of cancer proliferation. These views are in accordance with modern concepts of clinical oncology. GH-RH antagonists should be given alone or together with somatostatin analogues and a combination would achieve a more complete suppression of IGF-I levels, elimination of tissue IGF-I levels, e.g., in human osteosarcomas, as well as breast cancer, colon cancer, prostate cancer, and non-small cell lung cancer (non-SCLC).

The advantage of GH-RH antagonists over somatostatin analogues is based on the fact that GH-RH antagonists may be utilized for suppression of tumors which do not have somatostatin receptors, for example human

osteogenic sarcomas.

The present invention is described in connection with the following examples which are set forth for the purposes of illustration only. In the examples, optically active protected amino acids in the L-configuration are used except where specifically noted.

The following Examples set forth suitable methods of synthesizing the novel GH-RH antagonists by the solid-phase technique.

EXAMPLE I

Synthesis of Boc-agmatine

EXAMPLE II

Synthesis of 4-Chlorosulfonyl Phenoxyacetic Acid (Cl-SPA)

EXAMPLE III

Boc-agmatine-[SPA]

EXAMPLE IV

Coupling of Boc-agmatine-[SPA] to Support Phase

The initial synthetic sequence utilized herein and indicated by headings above is disclosed in Examples I through IV of US Patent 4,914,189, which Examples are incorporated herein by reference.

EXAMPLE V

Ibu-Tyr^{1}-D-Arg^{2}-Asp^{3}-Ala^{4}-Ile^{5}-Phe(pCl)^{6}-Thr^{7}-Asn^{8}-Ser^{9}-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-Cit^{29}-NH_2 (Peptide 1)

([Ibu^{0},D-Arg^{2},Phe(pCl)^{6},Abu^{15},Nle^{27},Cit^{29}]hGH-RH(1-29)NH_2)

The synthesisis is conducted in a stepwise manner using manual solid phase peptide synthesis equipment. Briefly, benzhydrylamine (BHA) resin (Bachem, California) (100 mg, 0.044 mmole) is neutralized with 5% DIEA in CH_2Cl_2 and washed according to the protocol described in Table 1. The

solution of Boc-Cit-OH (61mg, 0.25 mmole) in DMF-CH_2Cl_2 (1:1) is shaken with the neutralized resin and DIC (44 μL, 0.275 mmole) in a manual solid phase peptide synthesis equipment for 1 hour. After the completion of the coupling reaction is proved by negative ninhydrin test, deprotection with 50% TFA in CH_2Cl_2, and neutralization with 5% DIEA in CH_2Cl_2, the peptide chain is built stepwise by coupling the following protected amino acids in the indicated order on the resin to obtain the desired peptide sequence: Boc-Ser(Bzl)-OH, Boc-Nle-OH, Boc-Ile-OH, Boc-Asp(OcHx)-OH, Boc-Gln-OH, Boc-Leu-OH, Boc-Leu-OH, Boc-Lys(2-Cl-Z)-OH, Boc-Arg(Tos)-OH, Boc-Ala-OH, Boc-Ser(Bzl)-OH, Boc-Leu-OH, Boc-Gln-OH, Boc-Abu-OH, Boc-Leu-OH, Boc-Val-OH, Boc-Lys(2-Cl-Z)-OH, Boc-Arg(Tos)-OH, Boc-Tyr(2,6-diCl-Z)-OH, Boc-Ser(Bzl)-OH, Boc-Asn-OH, Boc-Thr(Bzl)-OH, Boc-Phe(pCl)-OH, Boc-Ile-OH, Boc-Ala-OH, Boc-Asp(OcHx)-OH, Boc-D-Arg(Tos)-OH, Boc-Tyr(2,6-diCl-Z)-OH.

These protected amino acid residues (also commonly available from Bachem Co.) are represented above according to a well accepted convention. The suitable protecting group for the side chain functional group of particular amino acids appears in parentheses. The OH groups in the above formulae indicate that each residue's carboxyl terminus is free.

The protected amino acids (0.25 mmole each) are coupled with DIC (44 μL, 0.275 mmole) with the exceptions of Boc-Asn-OH and Boc-Gln-OH which are coupled with their preformed HOBt esters. After removal of the N^{α}-Boc protecting group from Tyr^1, the peptide is acylated with isobutyric acid (Ibu) (75 mg, 0.4 mmole) using DIC (70 μL, 0.44 mmole).

In order to cleave the peptide from the resin and deprotect it, the dried peptide resin (138 mg) is stirred with 0.5 mL m-cresol and 5 mL hydrogen fluoride (HF) at 0°C for 1 hour. After evaporation of the HF under vacuum, the remnant is washed with dry diethyl ether and ethyl acetate.

The cleaved and deprotected peptide is dissolved in 50 % acetic acid and separated from the resin by filtration. After dilution with water and lyophilization, 34 mg crude product is obtained.

The crude peptide is checked by analytical HPLC using a Hewlett-Packard Model HP-1090 liquid chromatograph with a W-Porex C18 reversed-phase column (4.6 x 250mm, 5μm particle size, 300 Å pore size from Phenomenex, Rancho Palos Verdes, CA) and linear gradient elution, (e.g., 40-70% B) with a solvent system consisting of (A) 0.1% aqueous TFA and (B) 0.1% TFA in 70% aqueous MeCN. 34 mg of the crude peptide is dissolved in AcOH/H_2O, stirred, filtered and applied on a VYDAC 228TP column (10 x 250 mm) packed with C8 silica gel. The column is eluted with a solvent system described above in a linear gradient mode (e.g., 30-55% B in 120 min); flow rate 2mL/min. The eluent is monitored at 220 nm, and fractions are examined by analytical HPLC. Fractions with purity higher than 95% are pooled and lyophilized to give 1.2 mg pure product. The analytical HPLC is carried out on a W-Porex C18 reversed-phase column described above using isocratic elution with a solvent system described above with a flow rate of 1.2 mL/min. The peaks are monitored at 220 and 280 nm. The product is judged to be substantially (>95%) pure by analytical HPLC. The expected amino acid composition is also confirmed by amino acid analysis.

Peptides 2 and 3 are synthesized in the same manner as Peptide 1, except that Boc-Asn-OH^8 is replaced with Boc-Abu-OH (0.25 mmole) and the resulting peptides are acylated with isobutyric acid or phenylacetic acid repectively, to yield:

[Ibu^0,D-Arg^2,Phe(pCl)^6,Abu^{8,15},Nle^{27},Cit^{29}]hGH-RH(1-29)-NH_2 Peptide # 2

[PhAc^0,D-Arg^2,Phe(pCl)^6,Abu^{8,15},Nle^{27},Cit^{29}]hGH-RH(1-29)-NH_2 Peptide # 3

EXAMPLE VI

$PhAc^0$-Tyr^1-D-Arg^2-Asp^3-Ala^4-Ile^5-$Phe(pCl)^6$-Thr^7-Asn^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Abu^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-Agm^{29} (Peptide 8)

or [$PhAc^0$,D-Arg^2,$Phe(pCl)^6$,Abu^{15},Nle^{27}]hGH-RH(1-28)Agm

The synthesisis is conducted in a stepwise manner using manual solid phase peptide synthesis equipment. Briefly, Boc-Agm-SPA-aminomethyl resin (California Peptide Research Co., Inc., California) (200 mg, 0.06 mmole) is deprotected with 50% TFA in CH_2Cl_2, neutralized with 5% DIEA in CH_2Cl_2, and washed as described in Table I. The solution of Boc-Ser(Bzl)-OH (55 mg, 0.18 mmole) in DMF-CH_2Cl_2 (1:1) is shaken with the H-Agm-SPA-aminomethyl resin and DIC (31 μL, 0.2 mmole) in a manual solid phase peptide synthesis equipment for 1 hour. After the completion of the coupling reaction is proved by negative ninhydrin test, deprotection with 50% TFA in CH_2Cl_2 and neutralization with 5% DIEA in CH_2Cl_2, and washed as described in Table I to build the peptide chain step-wise by coupling the following protected amino acids in the indicated order on the resin: Boc-Nle-OH, Boc-Ile-OH, Boc-Asp(OcHx)-OH, Boc-Gln-OH, Boc-Leu-OH, Boc-Leu-OH, Boc-Lys(2-Cl-Z)-OH, Boc-Arg(Tos)-OH, Boc-Ala-OH, Boc-Ser(Bzl)-OH, Boc-Leu-OH, Boc-Gln-OH, Boc-Abu-OH, Boc-Leu-OH, Boc-Val-OH, Boc-Lys(2-Cl-Z)-OH, Boc-Arg(Tos)-OH, Boc-Tyr(2,6-diCl-Z)-OH, Boc-Ser(Bzl)-OH, Boc-Asn-OH, Boc-Thr(Bzl)-OH, Boc-Phe(pCl)-OH, Boc-Ile-OH, Boc-Ala-OH, Boc-Asp (OcHx)-OH, Boc-D-Arg(Tos)-OH and Boc-Tyr(2,6-diCl-Z)-OH.

The protected amino acids (0.18 mmole each) are coupled with DIC (31 μL, 0.2 mmole) with the exceptions of Boc-Asn-OH and Boc-Gln-OH which are coupled with their preformed HOBt esters. After removal of the N^{α}-Boc protecting group from Tyr^1, the peptide is acylated with phenylacetic acid (66 mg, 0.5 mmole) using DIC (88 μL, 0.55 mmole) as coupling agent.

In order to cleave the peptide from the resin and deprotect it, the dried peptide resin (148 mg) is stirred with 0.5 mL m-cresol and 5 mL hydrogen fluoride (HF) at 0°C for 1 hour. After evaporation of the HF under vacuum, the remnant is washed with dry diethyl ether and ethyl acetate. The cleaved and deprotected peptide is dissolved in 50% acetic acid and separated from the resin by filtration. After dilution with water and lyophilization, 62 mg crude product is obtained.

62 mg of the GH-RH antagonist peptide is dissolved in AcOH/H_2O and purified by RP-HPLC using the same procedure and equipments described in Example V. The product is judged to be substantially (>95%) pure by analytical HPLC. Confirmation of the structure is provided by amino acid analysis.

Peptides 4, 5, 6, 7, 15, 16, 25 and 26 are synthesized in the same manner as Peptide 1, except that these peptides also contain other substitutions, to yield:

Ac-Nal^0,D-Arg^2, Phe(pCl)^6, Abu^{15},Nle^{27}]hGH-RH(1-28)Agm

Peptide # 4

[PhAc^0,D-Arg^2, Phe(pCl)^6, Ala^{15},Nle^{27},Asp^{28}]hGH-RH(1-28)Agm

Peptide 5

[Ibu^0,D-Arg^2, Phe(pCl)^{6,10}, Abu^{15},Nle^{27}]hGH-RH(1-28)Agm

Peptide # 6

[Ibu^0,D-Arg^2, Phe(pCl)^6, D-Thr^8,Abu^{15},Nle^{27}]hGH-RH(1-28)Agm

Peptide 7

[PhAc^0,D-Arg^2, Phe(pCl)^6, Orn^{12,21}, Abu^{15}, Nle^{27}]hGH-RH(1-28)Agm

Peptide 15

[Ibu^0,D-Arg^2, Phe(pCl)^6, Orn^{12,21}, Abu^{15}, Nle^{27}]hGH-RH(1-28)Agm

Peptide 16

[PhAc^0, D-Arg^2, Tic^6, Abu^{15}, Nle^{27}) hGH-RH (1-28)Agm

Peptide 25

[PhAc^0, D-Tic^2, Phe(pCl)^6, Abu^{15}, Nle^{27}) hGH-RH (1-28)Agm
Peptide 26

EXAMPLE VII

PhAc-Tyr^1-D-Arg^2-Asp^3-Ala^4-Ile^5-Phe(pCl)^6-Thr^7-Asn^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Leu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-Arg^{29} -NH_2 (Peptide 9)
or [PhAc^0,D-Arg^2,Phe(pCl)^6,Ala^{15},Nle^{27}]hGH-RH(1-29)NH_2

The synthesis is conducted in a stepwise manner using manual solid phase peptide synthesis equipment. Briefly, benzhydrylamine (BHA) resin (Bachem, California) (100 mg, 0.044 mmole) is neutralized with 5% DIEA in CH_2Cl_2 and washed according to the protocol described in Table 1. The solution of Boc-Arg(Tos)-OH (107 mg, 0.25 mmole) in DMF-CH_2Cl_2 (1:1) is shaken with the neutralized resin and DIC (44 μL, 0.275 mmole) in a manual solid phase peptide synthesis equipment for 1 hour. After the completion of the coupling reaction is proved by negative ninhydrin test, deprotection with 50% TFA in CH_2Cl_2, and neutralization with 5% DIEA in CH_2Cl_2, the peptide chain is built stepwise by adding the following protected amino acids in the indicated order on the resin to obtain the desired peptide sequence: Boc-Ser(Bzl)-OH, Boc-Nle-OH, Boc-Ile-OH, Boc-Asp(OcHx)-OH, Boc-Gln-OH, Boc-Leu-OH, Boc-Leu-OH, Boc-Lys(2-Cl-Z)-OH, Boc-Arg(Tos)-OH, Boc-Ala-OH, Boc-Ser(Bzl)-OH, Boc-Leu-OH, Boc-Gln-OH, Boc-Ala-OH, Boc-Leu-OH, Boc-Val-OH, Boc-Lys(2-Cl-Z)-OH, Boc-Arg(Tos)-OH, Boc-Tyr(2,6-diCl-Z)-OH, Boc-Ser(Bzl)-OH, Boc-Asn-OH, Boc-Thr(Bzl)-OH, Boc-Phe(pCl)-OH, Boc-Ile-OH, Boc-Ala-OH, Boc-Asp(OcHx)-OH, Boc-D-Arg(Tos)-OH, Boc-Tyr(2,6-diCl-Z)-OH.

The protected amino acids (0.25 mmole each) are coupled with DIC (44 μL, 0.275 mmole) with the exceptions of Boc-Asn-OH and Boc-Gln-OH which are coupled with their preformed HOBt esters. After removal of the

N^{α}-Boc protecting group from Tyr^1, the peptide is acylated with phenylacetic acid (PhAc) (54 mg, 0.4 mmole) using DIC (70 μL, 0.44 mmole).

In order to cleave the peptide from the resin and deprotect it, the dried peptide resin (171 mg) is stirred with 0.5 mL m-cresol and 5 mL hydrogen fluoride (HF) at 0°C for 1 hour. After evaporation of the HF under vacuum, the remnant is washed with dry diethyl ether and ethyl acetate. The cleaved and deprotected peptide is dissolved in 50 % acetic acid and separated from the resin by filtration. After dilution with water and lyophilization, 98 mg crude product is obtained.

The crude peptide is checked by analytical HPLC using a Hewlett-Packard Model HP-1090 liquid chromatograph with a W-Porex C18 reversed-phase column (4.6 x 250mm, 5μm particle size, 300 Å pore size from Phenomenex, Rancho Palos Verdes, CA) and linear gradient elution, (e.g., 40-70% B) with a solvent system consisting of (A) 0.1% aqueous TFA and (B) 0.1% TFA in 70% aqueous MeCN. 60 mg of the crude peptide is dissolved in AcOH/H_2O, stirred, filtered and applied on a VYDAC 228TP column (10 x 250 mm) packed with C8 silica gel. The column is eluted with a solvent system described above in a linear gradient mode (e.g., 30-55% B in 120 min); flow rate 2mL/min. The eluent is monitored at 220 nm, and fractions are examined by analytical HPLC. Fractions with purity higher than 95% are pooled and lyophilized to give 2.6 mg pure product. The analytical HPLC is carried out on a W-Porex C18 reversed-phase column described above using isocratic elution with a solvent system described above with a flow rate of 1.2 mL/min. The peaks are monitored at 220 and 280 nm. The peptide is judged to be substantially (>95%) pure by analytical HPLC. The expected amino acid composition is also confirmed by amino acid analysis.

Peptides 10, 11, 12, 13, and 14 are synthesized in the same manner as Peptide 9, except that these peptides also contain other substitutions, to

yield:

[PhAc^0,Tyr(Me),D-Arg^2,Ala^{15}, Nle^{27}]hGH-RH(1-29)-NH$_2$

Peptide 10

[PhAc^0,D-Arg^2,Phe(pCl)^6,Abu^8,Ala^{15},Nle^{27}],hGH-RH(1-29)NH$_2$

Peptide 11

[PhAc^0,D-Arg^2, Nal^6, Abu^{8,28}, Ala^{15}, Nle^{27}]hGH-RH(1-29)NH$_2$

Peptide 12

[PhAc^0,D-Arg^2, Phe(pCl)^6, Abu^{8,28}, Ala^{15}, Nle^{27}]hGH-RH(1-29)NH$_2$

Peptide 13

[PhAc^0,D-Arg^2, Phe(pCl)^6, Abu^8, D-Lys^{12}, Ala^{15}, Nle^{27}]hGH-RH(1-29)NH$_2$

Peptide 14

EXAMPLE VIII

PhAc-Tyr^1-D-Arg^2-Asp^3-Ala^4-Ile^5-Phe(pCl)^6-Thr^7-Ser^8-Ser^9-Tyr^{10}-Arg^{11}-Lys^{12}-Val^{13}-Leu^{14}-Ala^{15}-Gln^{16}-Glu^{17}-Ser^{18}-Ala^{19}-Arg^{20}-Lys^{21}-Leu^{22}-Leu^{23}-Gln^{24}-Asp^{25}-Ile^{26}-Nle^{27}-Ser^{28}-Arg^{29}-NH$_2$ Peptide18

or cyclo^{17,21}[PhAc^0,D-Arg^2,Phe(pCl)^6,Ser^8,Ala^{15},Glu^{17},Nle^{27}]hGH-RH(1-29)NH$_2$

The synthesis is conducted in a stepwise manner using manual solid phase peptide synthesis equipment. Briefly, benzhydrylamine (BHA) resin (Bachem, California) (100 mg, 0.044 mmole) is neutralized with 5% DIEA in CH_2Cl_2 and washed according to the protocol described in Table 1. The solution of Boc-Arg(Tos)-OH (107 mg, 0.25 mmole) in DMF-CH_2Cl_2 (1:1) is shaken with the neutralized resin and DIC (44 μL, 0.2$^-$ mmole) in a manual solid phase peptide synthesis equipment for 1 hour. After the completion of the coupling reaction is proved by negative ninhydrin test, deprotection with 50% TFA in CH_2Cl_2, and neutralization with 5% DIEA in CH_2Cl_2, the peptide chain is built stepwise by coupling the following protected amino acids in the indicated order on the resin to obtain the desired peptide sequence: Boc-Ser(Bzl)-OH, Boc-Nle-OH, Boc-Ile-OH, Boc-Asp(OcHx)-OH, Boc-Gln-OH, Boc-

Leu-OH, Boc-Leu-OH, Boc-Lys(Fmoc)-OH, Boc-Arg(Tos)-OH, Boc-Ala-OH, Boc-Ser(Bzl)-OH, Boc-Glu(Fm)-OH, Boc-Gln-OH, Boc-Ala-OH, Boc-Leu-OH, Boc-Val-OH, Boc-Lys(2-Cl-Z)-OH, Boc-Arg(Tos)-OH, Boc-Tyr(2,6-diCl-Z)-OH, Boc-Ser(Bzl)-OH, Boc-Ser(Bzl)-OH, Boc-Thr(Bzl)-OH, Boc-Phe(pCl)-OH, Boc-Ile-OH, Boc-Ala-OH, Boc-Asp(OcHx)-OH, Boc-D-Arg(Tos)-OH, Boc-Tyr(2,6-diCl-Z)-OH.

The protected amino acids (0.25 mmole each) are coupled with DIC (44 μL, 0.275 mmole) with the exceptions of Boc-Asn-OH and Boc-Gln-OH which are coupled with their preformed HOBt esters. After removal of the N^{α}-Boc protecting group from Tyr^{1}, the peptide is acylated with phenylacetic acid (PhAc) (75 mg, 0.4 mmole) using DIC (70 μL, 0.44 mmole). The acylated peptide is deprotected with 20% piperidine in DMF for 1 plus 20 minutes to give free side-chains of Glu^{17} and Lys^{21}. After washing the resin, lactam bridge is formed with HBTU reagent (110 mg, 0.275 mmole) in DMF-DCM (1:1) containing DIEA (137 μL, 0.55 mmole) and HOBt (39 mg, 0.275 mmole) for 3 hours (negative Kaiser ninhydrin test).

In order to cleave the peptide from the resin and deprotect it, the dried peptide resin (53.1 mg) is stirred with 0.5 mL m-cresol and 5 mL hydrogen fluoride (HF) at 0°C for 1 hour. After evaporation of the HF under vacuum, the remnant is washed with dry diethyl ether and ethyl acetate. The cleaved and deprotected peptide is dissolved in 50 % acetic acid and separated from the resin by filtration. After dilution with water and lyophilization, 13.7 mg crude product is obtained.

The crude peptide is checked by analytical HPLC using a Hewlett-Packard Model HP-1090 liquid chromatograph with a W-Porex C18 reversed-phase column (4.6 x 250mm, 5μm particle size, 300 Å pore size from Phenomenex, Rancho Palos Verdes, CA) and linear gradient elution, (e.g., 40-70% B) with a solvent system consisting of (A) 0.1% aqueous TFA and

(B) 0.1% TFA in 70% aqueous MeCN. The crude peptide is dissolved in AcOH/H_2O, stirred, filtered and applied on a VYDAC 228TP column (10 x 250 mm) packed with C8 silica gel. The column is eluted with a solvent system described above in a linear gradient mode (e.g., 30-55% B in 120 min); flow rate 2mL/min. The eluent is monitored at 220 nm, and fractions are examined by analytical HPLC. Fractions with purity higher than 95% are pooled and lyophilized to give 0.96 mg pure product. The analytical HPLC is carried out on a W-Porex C18 reversed-phase column described above using isocratic elution with a solvent system described above with a flow rate of 1.2 mL/min. The peaks are monitored at 220 and 280 nm. The peptide is judged to be substantially (>95%) pure by analytical HPLC. The expected amino acid composition is also confirmed by amino acid analysis.

Peptides 17, 19 and 20 are synthesized in the same manner as Peptide 18, except that in addition to other substitutions lactam bridge is formed between positions 8,12 or 25,29, to yield:

cyclo^{8,12}[PhAc^0, D-Arg^2, Phe(pCl)^6, Glu^8, Ala^{15}, Nle^{27}]hGH-RH(1-29)NH_2 Peptide # 17

cyclo^{25,29}[PhAc^0,D-Arg^2,Phe(pCl)^6,Abu^8,Ala^{15},D-Asp^{25},Nle^{27},Orn^{29}]hGH-RH(1-29)NH_2 Peptide # 19

cyclo^{25,29}[PhAc^0,D-Arg^2,Nal^6,Abu^8, Ala^{15}, D-Asp^{25},Nle^{27},Orn^{29}]hGH-RH(1-29)NH_2 Peptide # 20

Peptides 21, 22, 23, and 24 are synthesized in the same manner as Peptide 18, except that in addition to other substitutions lactam bridge is formed between both 8,12 and 1,25 positions, to yield:

cyclo $^{8,12;21,25}$[PhAc^0, D-Arg^2, Phe(pCl)^6, Glu^{8,25}, Abu^{15}, Nle^{27}]hGH-RH(1-28)Agm Peptide #21

cyclo $^{8,12;21,25}$[PhAc^0, D-Arg^2, D-Asp^3, Phe(pCl)^6, Glu^{8,25}, D-Lys^{12}, Ala^{15}, Nle^{27}]hGH-RH(1-29)NH_2 Peptide # 22

cyclo $^{8, 12; 21,25}$[PhAc^0, D-Arg^2, Phe(pCl)^6, Glu$^{8, 25}$, D-Lys^{12}, Ala^{15}, Nle^{27}]hGH-RH(1-29)NH_2 Peptide # 23

cyclo $^{8, 12; 21,25}$[PhAc^0, D-Arg^2, Phe(pCl)^6, Asp^8, D-Lys^{12}, Ala^{15}, Glu^{25}, Nle^{27}]hGH-RH(1-29)NH_2 Peptide # 24

EXAMPLE IX

Biological Activity

The peptides of the present invention were tested in an in vitro and in vivo assay for their ability to inhibit the hGH-RH(1-29)NH_2 induced GH release.

Superfused Rat Pituitary System. The analogues were tested in vitro in a test described earlier (S. Vigh and A.V. Schally, Peptides 5:241-347, 1984) with modification (Z. Rekasi and A.V. Schally, P.N.A.S. 90:2146-2149, 1993).

Briefly, the cells are preincubated with peptides for 9 minutes (3mL) at various concentrations. Immediately after the incubation, 1 nM hGH-RH(1-29)NH_2 is administered for 3 minutes (1mL) [0 minute response]. To check the duration of the antagonistic effect of the analogue, 1 nM hGH-RH(1-29)NH_2 is applied 30, 60, 90, and 120 minutes later for 3 minutes [30, 60, 90, 120 min responses]. Net integral values of the GH responses are evaluated. GH responses are compared to and expressed as percent of the original GH response induced by 1 nM GH-RH(1-29)NH_2. The effect of the new antagonists are compared to that of [Ac-Tyr^1,D-Arg^2]hGH-RH(1-29)NH_2, the "Standard antagonist".

Growth Hormone Radio-immunoassay. Rat GH levels in aliquots of undiluted and diluted superfusion samples were measured by double-antibody radioimmunoassay using materials supplied by the National Hormone and Pituitary Program, Baltimore, Maryland. The results of RIA

were analyzed with a computer program developed in our institute (V. Csernus and A.V. Schally, Harwood Academic (Greenstein, B.C. ed., London, pp. 71-109, 1991), hereby incorporated by reference. Inter-assay variation was less than 15% and intra-assay variation was less than 10%.

GH-RH Binding Assay. A sensitive radioreceptor binding assay was developed to determine the binding characteristics of the antagonists of GH-RH (G. Halmos, A.V. Schally et al., Receptor 3, 87-97, 1993), hereby incorporated by reference. The assay is based on binding of labelled [His^1,Nle^{27}]hGH-RH(1-32)NH_2 to rat anterior pituitary membrane homogenates. Iodinated derivatives of [His^1,Nle^{27}]hGH-RH(1-32)NH_2 are prepared by the chloramine-T method (F.C. Greenwood et al., Biochemistry 89:114-123, 1963), hereby incorporated by reference. Pituitaries from male Sprague-Dawley rats (250-300 g) are used to prepare crude membranes. For saturation binding analyses, membrane homogenates are incubated with at least 6 concentrations of [His^1,^{125}I-Tyr^{10},Nle^{27}]hGH-RH(1-32) NH_2, ranging from 0.005 to 0.35 nM in the presence or absence of excess unlabelled peptide (1 μM).

The pellet is counted for radioactivity in a γ-counter. The affinities of the antagonist peptides tested to rat pituitary GH-RH receptors are determined in competitive binding experiments. The final binding affinities are estimated by K_i (dissociation constant of the inhibitor-receptor complex) and are determined by the Ligand PC computer program of Munson and Rodbard as modified by McPherson. Relative affinities compared to [Ac-Tyr^1,D-Arg^2]hGH-RH(1-29)NH_2, the Standard antagonist, are calculated as the ratio of K_i of the tested GH-RH antagonist to the K_i of the Standard antagonist.

In Vivo Tests. Adult male Sprague-Dawley rats are anesthetized with pentobarbital (6mg/100g b.w., i.p.). Blood samples are taken from the

jugular vein 30 min after the injection of pentobarbital. One group of 7 animals receives hGH-RH(1-29)NH_2 as control. Other groups of rats are injected with [Ac-Tyr^1,D-Arg^2]hGH-RH(1-29) NH_2 as Standard antagonist, or with one of the antagonist peptide 30 seconds prior to hGH-RH (1-29)NH_2, which is administered at dose of 2-3 μg/kg b.w. Blood samples are taken from the jugular vein 5 and 15 min after the injection of antagonists. GH levels are measured by RIA. Potencies of the antagonists are calculated by the factorial analysis of Bliss and Marks with 95% confidence limits and are based on the doses of 100 and 400 μg/kg b.w. of the Standard antagonist and 20 and 80 μg/kg b.w. of the antagonists tested. Statistical significance was assessed by Duncan's new multiple range test.

Results in vitro. The results of the in vitro antagonistic activities tested in superfused rat pituitary system and binding assay are summarized in Table II and Table III, respectively. As can be seen from these data, acylation of the analogs with PhAc or Ibu which contain D-Arg^2 substitution combined with $Phe(pCl)^6$ or Nal^6, Abu^8, Abu^{15} or Ala^{15}, Nle^{27}, Asp^{28}, Agm^{29} or Arg^{29}-NH_2, and/or lactam bridge cause an immense increase in receptor binding as well as in inhibition of GH release in vitro and in vivo. Antagonists[$PhAc^0$,D-Arg^2,pCl-Phe^6,Abu^{15},Nle^{27}]hGH-RH(1-28)Agm(MZ-5-156), [$PhAc^0$-,D-Arg^2,$Phe(pCl)^6$,$Abu^{8,28}$,Ala^{15},Nle^{27}]hGH-RH(1-29)NH_2(MZ-5-208), [$PhAc^0$-,D-Arg^2,$Phe(pCl)^6$,Abu^8,Ala^{15},Nle^{27}]hGH-RH(1-29)NH_2 (MZ-5-192), [$PhAc^0$,D-Arg^2,$Phe(pCl)^6$,Ala^{15},Nle^{27},Asp^{28}]hGH-RH(1-28)Agm(MZ-5-116), and cyclo ^{17,21} [$PhAc^0$,D-Arg^2,$Phe(pCl)^6$,Ser^8,Ala^{15},Glu^{17},Nle^{27}]hGH-RH(1-29)NH_2 are the most effective antagonists in vitro.

Results in vivo. Table IV shows the serum GH levels in rats pretreated with GH-RH antagonists. Peptides 7, 8, 9, 11, 13, and 18 produce a significant greater and longer-lasting inhibition of the GH release stimulated by hGH-RH(1-29)NH_2 than the standard antagonist. In vivo experiments, Peptide 8 (MZ-5-156) inhibits hGH-RH(1-29)NH_2-induced GH-release to greater extent than MZ-4-71,[Ibu^0,D-Arg^2,Phe(pCl)^6,Abu^{15},Nle^{27}] hGH-RH(1-28)Agm, the previously most effective antagonist.

TABLE II

Inhibition of GH Release in Superfused Rat Pituitary System

Peptide	Dose (nM)	Inhibition of GH release (%) 0 min	30 min	60 min	90 min	120 min
Standard antagonist:	100	62.1	2.5	19		
Peptide 1	3	60.1	12.4	11.5		
	30	98	49.2	37.6		
Peptide 2	3	27.4	16.2	48.4	30.2	
	30	94.7	53.0	24.4	41.8	
Peptide 3	30	97.4	32.2	35.9	42.1	
Peptide 4	3	28	21.3	15		
	30	76.7	53.4	32		
Peptide 5	30	97.1	93.9	80.1	56.9	
Peptide 6	30	86.3	89.9	78.9	87.6	62.7
Peptide 7	30	96.3	41.5	21.5	23.2	20.6
Peptide 8	3	78.8	73.3	65.9	54.9	
	10	88.8	83.5	64.9	51.7	42.3
	30	94.5	90.0	71.5	65.0	55.6
Peptide 9	30	92.0	80.6	58.9	46.7	46.0
Peptide 10	30	84.8	35.2	38.2	29.8	33.8
Peptide 11	30	92.3	80.9	73.3	63.2	55.7
Peptide 15	30	85.5	65.2	56.1	44.0	46.7
Peptide 16	30	85.2	12.3	25.1	14.8	36.0
Peptide 12	30	87.0	61.0	37.0	34.0	41.0
Peptide 13	30	92.3	90.0	77.3	64.7	61.1
Peptide 14	30	87.0	32.0	6.0	12.0	24.0
Peptide 17	30	87.3	46.4	10.7	17.4	
Peptide 18	30	96.8	79.4	68.3	47,1	
Peptide 21	30	79.6	56.5	47.5	53.4	
Peptide 22	30	65.8	37.7	24.9	26.8	
Peptide 23	30	54.5	28.3	16.3	21.0	
Peptide 19	30	17.5	0.0	11.2	14.0	
Peptide 20	30	11.0	0.0	0.0	13.0	
Peptide 24	30	46.0	6.0	26.0	31.0	

TABLE III

K_i values and relative affinities (R.A) of hGH-RH antagonists

Peptide	K_i (nM)*	R.A.
Standard	3.37 ± 0.14	1
Peptide 3	0.301 ± 0.14	11.19
Peptide 5	0.096	35.10
Peptide 8	0.0159 ± 0.01	48.84
Peptide 18	0.159 ± 0.04	21.19
Peptide 11	0.059 ± 0.001	57.12
Peptide 23	0.456	7.39

TABLE IV

Serum Growth Hormone Levels in Rats Pretreated with Different GH-RH Antagonists at Different Time Intervals Prior to GH-RH(1-29)NH_2 at a Dose of 3 μg/kg

Treatment Inhibition (intravenously)		Dose (μg/kg)	Serum GH Levels (ng/mL)	%
GH-RH(1-29)NH_2		3.0	695.15 ± 95.40	0%
Peptide 18	2 min	80	370.31 ± 48.26	47%··
	15 min	80	564.74 ± 76.99	N.S.
Peptide 9	2 min	80	450.21 ± 68.38	35%
GH-RH(1-29)NH_2		3.0	703.34 ± 93.01	0%
Peptide 8	2 min	80	177.13 ± 31.75 ··	75%
	15 min	80	183.91 ± 26.19 ··	74%
GH-RH(1-29)NH_2		3.0	724.68 ± 93.01	0%
Peptide 8	30 min	80	567.46 ± 50.70	22% NS
	60 min	80	582.41 ± 70.18	20% NS
GH-RH(1-29)NH_2		3.0	883.36 ± 148.34	0%
Peptide 13	2 min	80	316.01 ± 68.24 ··	64%
	15 min	80	641.73 ± 92.01 ·	27%
GH-RH(1-29)NH_2		3.0	703.34 ± 93.01	0%
Peptide 7	2 min	80	611.07 ± 87.75 ·	31%
	15 min	80	688.39 ± 107.91	N.S.
GH-RH(1-29)NH_2		3.0	724.68 ± 93.01	0%
Peptide A	15 min	80	585.33 ± 89.34 ·	43%
	30 min	80	788.39 ± 96.93	23% NS

Data are expressed as mean ± SEM · $p < 0.05$ vs control
·· $p < 0.01$ vs control NS not significant

Effect of GH RH antagonists on SW 1990 human pancreatic cancers in nude mice

Small piees of an SW-1990 rumor growing in a nude mouse were transplated s.c. into the right flanks of 60 nu/nu male nude mice. Ten days after transplantation, 40 mice with well growing tumors were groupled into 4 groups with equal average tumor volume (16mm^3) in each group. Treatment started 13 days after transplantation and was continuted for 44 days. Groups are shown in the table. The tumors were measured and volume was calculated weekly. Tumor volume changes are shown in Table VI. On day 45 of treatment the mice were sacraficed under Metofane anesthesia by exsanguination from abdominal aorta. Blood was collected, tumor and organ weights were determined.

Histology, receptor studies and determination of blood hormone levels are in progress.

Results

Tumor volume data and tumor weights at the end of the experiment are shown in the table. Treatment with 10 μg/day of Peptide #8 resulted in a significant inhibition of growth of SW-1990 cancers as shown by volume and weigth data. Peptide A had no significant effect on the tumors. Body, liver, kidney and spleen weights showed no significant differences from control values.

Effect of treatment with GH-RH antagonists on tumor weights and final tumor volume of SW-1990 humane pancreatic cancers growing in nude mice.

TABLE V

Group No.	Treatment	Dose mg/day	Number of mice	Tumor weights (mg)	Final Tumor Volume mm^3
1	Control		10	1467 ± 374	1291 ± 301
2	Peptide A	10	10	1467 ± 595	1183 ± 342
3	Peptide A	40	10	1297 ± 258	902 ± 156
4	Peptide 8	10	10	931 ± 330	612 ± 220*

*$p < 0.05$

Peptide A: [Ibu^0-D-Arg^2-Phe(pCl)^6-Abu^{15}-Nle^{27}] hGHRH (1-28) Agm

CLAIMS

1. A peptide selected from the group having the formulae:

α) X-R^1-R^2-R^3-R^4-R^5-R^6-Thr-R^8-R^9-R^{10}-R^{11}-R^{12}-Val-Leu-R^{15}-Gln-Leu-Ser-R^{19}-R^{20}-R^{21}-Leu-Leu-Gln-Asp-Ile-R^{27}-R^{28}-R^{29}

wherein X is H, Ac, Aqc, IAc, BrProp, For, Ibu, Nac, 2-Nac, 1- or 2-Npt, 1- or 2-Npr, PhAc, Fpr, or any other aromatic or nonpolar acyl group,

R^1 is Tyr, His or Phe(Y), in which Y = H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3,

R^2 is D-Arg, D-Cit, D-Har, D-Lys,D-Tic or D-Orn,

R^3 is Asp, D-Asp, Ala, D-Ala or Gly,

R^4 is Ala, Abu or Gly,

R^5 is Ile, Ala or Gly,

R^6 is Phe, Tic, Ala, Pro, Tpi, Nal or Phe(Y), in which Y = H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3,

R^8 is Asn, Gln, Ser, Thr, Val, Leu, Ile, Ala, D-Ala, D-Asn, D-Gln, D-Thr, D-Leu, Abu, D-Abu, Nle, or Aib,

R^9 is Ser,

R^{10} is Tyr or Phe(Y), in which Y = H, F, Cl, Br, NO_2, CH_3 or OCH_3,

R^{11} is Arg, D-Arg, or Cit,

R^{12} is Lys, D-Lys, Cit, D-Cit, Orn, D-Orn, Nle or Ala,

R^{15} is Gly, Ala, Abu or Gln,

R^{19} is Ala or Abu,

R^{20} is Arg, D-Arg or Cit,

R^{21} is Lys, D-Lys, Orn or Cit,

R^{27} is Met, Nle or Abu,

R^{28} is Ser, Asn, Asp, Ala or Abu,

R^{29} is Agm, Arg-NH_2, Arg-OH, Cit-NH_2, Cit-OH, Har-NH_2 or Har-OH, and

β) X-A^1-B^2-A^3-R^4-R^5-R^6-Thr-A^8-Ser-R^{10}-R^{11}-B^{12}-Val-Leu-R^{15}-A^{16}-A^{17}-Ser-R^{19}-B^{20}-B^{21}-Leu-Leu-Gln-A^{25}-Ile-R^{27}-R^{28}-B^{29}

wherein a lactam bridge is formed between any one of the pairs of positions 1,2; 2,3; 8,12; 16,20; 17-21; 21,25; 25,29 or both 8,12 and

21,25 positions and

X is H, Ac, Aqc, IAc, BrProp, For, Ibu, Nac, 2-Nac, 1- or 2-Npt, 1- or 2-Npr, PhAc, Fpr, or any other aromatic or nonpolar acyl group,

A is Glu, D-Glu, Gln, Asp, D-Asp, Asn, Abu, Leu, Tyr, His, Phe(Y), in which Y = H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3, Ser, Thr, Val, Ile, Ala, D-Ala, D-Asn, D-Gln, D-Thr, D-Leu, Abu, D-Abu, Nle, or Aib,

B is Lys, D-Lys, Arg, D-Arg, Orn, D-Orn, or Agm

R^4 is Ala, Abu or Gly,

R^5 is Ile, Ala or Gly,

R^6 is Phe, Tic, Tpi, Nal or Phe(Y), in which Y = H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3,

R^{10} is Tyr or Phe(Y), in which Y = H, F, Cl, Br, NO_2, NH_2, CH_3 or OCH_3,

R^{15} is Gly, Ala, Abu or Gln,

R^{27} is Met, Nle or Abu,

R^{28} is Ser, Asn, Asp, Ala or Abu,

and pharmaceutically acceptable salts thereof.

2. A compound of claim 1 selected from the group consisting of

[$PhAc^0$,D-Arg^2, Phe(pCl)^6, Ala^{15}, Nle^{27}, Asp^{28}]hGH-RH(1-28)Agm

Peptide 5

[Ibu^0,D-Arg^2, Phe(pCl)$^{6, 10}$, Abu^{15}, Nle^{27}]hGH-RH(1-28)Agm

Peptide 6

[$PhAc^0$,D-Arg^2, Phe(pCl)^6, Abu^{15}, Nle^{27}]hGH-RH(1-28)Agm

Peptide 8

[$PhAc^0$,D-Arg^2, Phe(pCl)^6, Ala^{15}, Nle^{27}]hGH-RH(1-29)-NH_2

Peptide 9

[$PhAc^0$,D-Arg^2,Phe(pCl)^6,Abu^8,Ala^{15},Nle^{27}]hGH-RH(1-29)NH_2

Peptide 11

[$PhAc^0$,D-Arg^2, Phe(pCl)^6, $Abu^{8, 28}$, Ala^{15}, Nle^{27}]hGH-RH(1-29)-NH_2

Peptide 13

3. A compound of claim 1 selected from the group consisting of
cyclo^{8,12}[PhAc^0,D-Arg^2,Phe(pCl)^6,Glu^8,Ala^{15},Nle^{27}]hGH-RH(1-29)-NH_2 Peptide 17
cyclo^{17,21}[PhAc^0,D-Arg^2,Phe(pCl)^6,Ser^8,Ala^{15},Glu^{17},Nle^{27}]hGH-RH(1-29)-NH_2 Peptide 18 (5-174)
cyclo$^{8,12;\ 21,25}$[PhAc^0,D-Arg^2,Phe(pCl)^6,Glu^{8,25},Abu^{15},Nle^{27}]
hGH-RH(1-28)Agm Peptide 21
cyclo$^{8,12;\ 21,25}$[[PhAc^0,D-Arg^2,D-Asp^3,Phe(pCl)^6,Glu^{8,25},D-Lys^{12}, Ala^{15}, Nle^{27}]hGH-RH(1-29)-NH_2 Peptide 22
cyclo$^{8,12;\ 21,25}$[[PhAc^0,D-Arg^2, Phe(pCl)^6, Glu$^{8,\ 25}$, D-Lys^{12}, Ala^{15}, Nle^{27}]
hGH-RH(1-29)-NH_2 Peptide 23

4. A method of suppressing excessive levels of GH in a patient in need of same which comprises administering said patient an effective amount of a compound of claim 1.

5. A method of treating a patient having a cancer carrying receptors for IGF-I which comprises administering to said patient an effective amount of a compound of claim 1.

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Volume changes of SW-1990 human pancreatic cancers in nude mice during treatment with antagonists of GH-RH (KS-51 experiment)

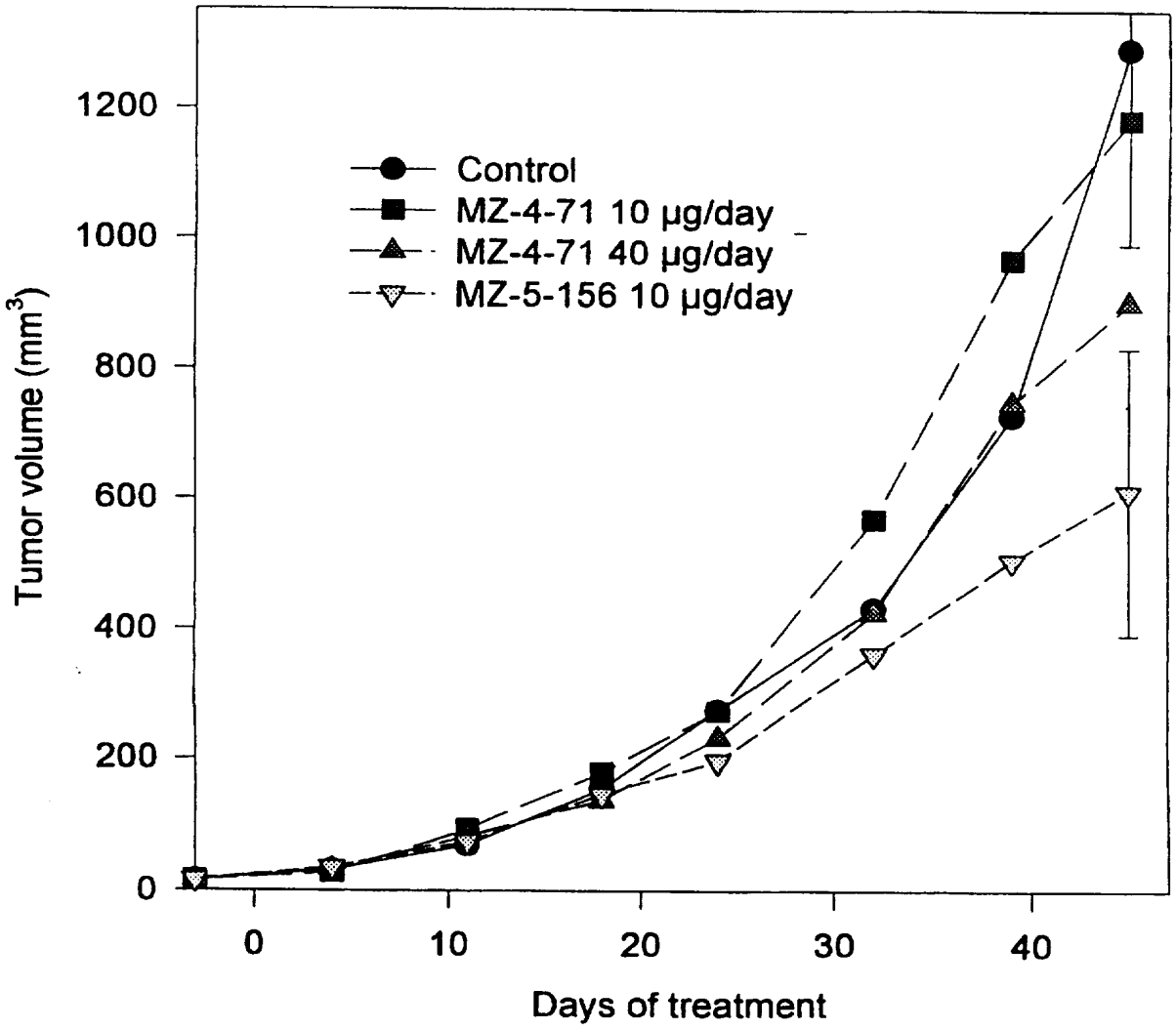

FIGURE 1

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/US 97/07452

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/60 A61K38/25

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 95 16707 A (UNIV TULANE ;SCHALLY ANDREW VICTOR (US); ZARANDI MARTA (US)) 22 June 1995 see the whole document ---	1,2,4,5 1,3-5
X Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, December 1994, WASHINGTON US, pages 12298-12302, XP002040642 ZARANDI E.A.: "Synthesis and biological activities of highly potent antagonists of GHRH" see the whole document ---	1,2,4,5 1,3-5
Y	EP 0 365 779 A (HOFFMANN LA ROCHE) 2 May 1990 cited in the application see the whole document --- -/--	1,3-5

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
15 September 1997	01. 10. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Groenendijk, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 97/07452

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 352 014 A (SALK INST FOR BIOLOGICAL STUDI) 24 January 1990 see the whole document ---	1,3-5
P,X	PEPTIDES, vol. 18, no. 3, 1997, pages 423-430, XP002040643 ZARANDI E.A.: "Synthesis and in vitro evaluation of new potent antagonists of GHRH" see the whole document ---	1,2,4,5
P,X	PEPTIDES, vol. 18, no. 3, 1997, pages 431-438, XP002040644 KOVACS E.A.: "Inhibition og GH release in rats by new potent antagonists of GHRH" see the whole document -----	1,2,4,5

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/07452

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 4, 5
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

Please see Further Information sheet enclosed.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

The formula in claim 1 under β (page 43, lines 28-29) is not completely defined: a definition of the variables R11 and R19 is lacking.
It is true that the formula in claim 1 under α also contains variables R11 and R19. However the corresponding variables R6 and R10 in both formula's are defined differently. Already for this reason it is not obvious to assume that said variables R11 and R19 would have the same meaning in both formula's.
Consequently the search could only encompass the meanings of the variables R11 and R19 as defined for the formula under β in the description on page 9, lines 1-9, that is R11 is Arg and R19 is Ala or Abu.

Incomplete Search:
Claims searched completely: 2, 3
Claims searched incompletly: 1, 4, 5

INTERNATIONAL SEARCH REPORT

...ıormation on patent family members

Interna l Application No
PCT/US 97/07452

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		JP 2069499 A	08-03-90